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Appn. Filed: 12/05/2001
Appn. #: 10/679,305
Applicant: Elizabeth Gay Frayne
Title: "Manipulation of RNA Stability and Protein Synthesis Using Thio-phosphate"
Examiner: Nancy T. Vogel
Art Unit:1636

Assistant Commissioner for Patents
Arlington, Virginia, 22313

RE: Office Action 6/16/11

I have prepared two continuation-in-part applications and filed them. One continuation elects claim nine and is titled "Method to Label Nucleic Acids in Cells In Vivo or In Vitro Using Thio-phosphate". Therefore, I think claim nine should be cancelled in the present application but I show the current modification of the claim in the present application. The other continuation-in-part application has the same title as the present application and does present some additional data in support of the application, particularly claim 10 and its dependent parts. As the examiner has allowed claim 8, I wish to have the present application issued with this claim.

Claim 10

There seems to be some confusion over the use of the word "induction". The dictionary defines this as the initiation or cause of a change or process. In the present case the latter definition of the word is intended. In fact the term is commonly used with cycloheximide induction even though cycloheximide inhibits protein synthesis. The change in the present invention is the creation of nuclease resistant linkages in mRNA leading to their stability and enhanced accumulation. Conditions were presented for optimal induction of mRNA. Since clearly more mRNA can be anticipated to produce more protein conditions were also described for this function of the invention. They are not entirely equivalent as optimal protein synthesis occurs with less than 100% modification of the mRNA backbone. To enhance protein synthesis one needs to measure this. In the present application this was done with enzymatic assays or via immunoprecitipation.

The present invention defines a specific chemical modification in RNA, namely, the creation of phosphorothioate linkages which are known to resist nuclease degradation in vivo and in vitro. The key of the present invention is the ability to create this chemical modification in vivo. One can control the extent of nucleic acid modification by simply varying the ratio of thio-phosphate to phosphate in the culture media. Thio-phosphate as a phosphate analogue is readily incorporated by RNA polymerase during the synthesis of all mRNAs. Upon induction or the addition of thio-phosphate into the culture media it will preferentially accumulate in those mRNAs that naturally turnover at a higher rate. It may also benefit unstable mRNAs more as stable mRNAs are inherently stable. At equilibrium all mRNAs are enhanced, being protected from nuclease degradation. For optimal protein synthesis though, media that is 100% substituted is not the desired condition even though this would lead to the maximal amount of mRNA. When cells make too much protein they form inclusion bodies in the case of bacteria or they secrete excess proteins and/or aggregate and precipitate them in the case of yeast.

The ability to stabilize mRNA is a known method to enhance protein synthesis. Claim 10 is directed at manipulating the protein synthesis of mRNAs in cell culture systems and not complex organisms. Cell culture systems allow ready control of the media concentration of thio-phosphate for sustained protein production. Not all proteins are equally enhanced to the same degree. In fact some proteins are decreased in amount. The extent of enhancement is related to the relative stability of the mRNA species in question. Thio-phosphate does not target specific species of mRNAs but rather influences bulk populations in regards to their protein synthesis. Induction of protein synthesis was indeed measured by comparing the amount of protein synthesis or enzyme activity at different ratios of thio-phosphate to phosphate. The control for such experiments is 0% thio-phosphate.

While the present comments may not overcome all of the examiner's objections, the continuation-in part application should help.

I wish to declare and make a verified statement that the modified claims include no new matter as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b) or 1.825 (d).

Respectfully submitted,

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